Chapter 14
Extracts from Fly Maggots and Fly Pupae as a "Wound Healer"

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Abstract On one hand the population in good old Europe and North America is decreasing in number, yet on the other there is an enormous increase in age. This leads to an increase in typical diseases of the elderly such as diabetes, decubitus and/or "nonhealing wounds" of other origin as consequences of incorrect diet and/or being constantly bedridden. There are many approaches to clear the situation of those people with "nonhealing wounds". However, although huge amounts of money are spent on different therapies thousands of amputations have to be carried out per year in most of the so-called industrialized countries. For example, in 2004 about 42,000 amputations were carried out in Germany as consequences of the typical diabetic-foot-syndrome that occurred among the 6-8 million people suffering in Germany from diabetes – worldwide there are more than 300 million humans involved in diabetic diseases. The chapter describes a new approach to heal "non-healing" wounds by use of a patented extract from larvae of the fly *Lucilia sericata*, which is finally lyophilyzed and thus can be stored for long before use as "wound cover".

14.1 Introduction

On one hand the population in good old Europe and North America is decreasing in number, yet on the other there is an enormous increase in age. This leads to an increase in typical diseases of the elderly such as diabetes, decubitus and/or "non-healing wounds" of other origin as consequences of incorrect diet and/or being constantly bedridden. There are many approaches to clear the situation of those people with "nonhealing wounds." However, although huge amounts of money are spent on different therapies thousands of amputations have to be carried out per year in most of the so-called industrialized countries. For example, in 2004 about 42,000 amputations were carried out in Germany as consequences of the typical diabetic-foot-syndrome that occurred among the 6–8 million people suffering in

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Germany from diabetes – worldwide there are more than 300 million humans involved in diabetic diseases.

Therefore, there is an increasing urgency to alleviate the suffering of the mostly very old patients living with such very painful and perspectiveless wounds.

One of the recent approaches besides different applications of mechanical and chemotherapeutic dressing of the wounds is the use of fly maggots as "biosurgeons" to clean wounds and thus to stimulate them to heal again (Baer 1931; Bonn 2000; Beasley and Hirst 2004; Bexfield et al. 2004, 2008, 2010; Evans 2002; Fleischmann and Grassberger 2002; Grassberger and Frank 2003; Hobson 1931; Horobin et al. 2003, 2005; Mumcuoglu 2001; Robinson and Norwood 1933; Sherman et al. 2000; Sherman 2003). Another approach entails the evaluation of different extracts of fly larvae for their ability to do the same as living fly maggots do, but with less risk of the transmission of bacterial superinfections and/or with less psychological stress for the mostly very old patients.

These approaches, although not new, but already known in various forms for centuries, are in a way astonishing as flies have been known for a very long time as vectors of agents of disease (Tables 14.1–14.3; Mehlhorn et al. 2010). Thus, contact of humans and their animals with flies has been intensively avoided by the development of skilful hygiene methods and a broad spectrum of insecticides that should reduce the number of flies in the surroundings of humans and their animals (Mehlhorn 2008).

Considering the fact, that flies which feed on living or dead bodies and/or live in or on decomposing material, had already solved problems with wound healing and defense against attacking microbes millions of years ago, it becomes more likely, that their "abilities and capacities" might be helpful to humans, too. Thus, it is therefore not surprising that the activities of flies had excited the interest of scientists as was seen 80 years ago by the discovery of fungi that produce antibiotics.

14.2 The Process of Wound Healing

The wound-healing process is based on many components that have to be active at particular phases during normal wound healing. Therefore, it is not surprising that – if one or several of these factors are disturbed or even completely blocked – wound healing might become severely affected. Principally two basic types of wound healing are distinguished (Knapp and Hansis 1999; Protz 2009).

14.2.1 The Primary Healing Wound

This type is seen in aseptic surgical wounds that form fresh (4–6 h old), noninfected wounds with rather close borders (e.g., knife cuts). These wounds are mostly closed at present within 8–10 days and only small scars remain visible.

Table 14.1 Developmental data of flies attacking man and animals

Species	Size/ adult (mm)	Eggs/place of deposition	Hatch of larvae within	Larval development	Pupal rest	Life span of adults
Musca domestica, house typhoid fly	6–7	600–1,000 of 0.25 mm on feces	15°C: 50 h 20°C: 23 h 30°C: 10 h	15°C: 10 Days 20°C: 8 Days 30°C: 4 Days	15°C: 18 Days 20°C: 10 Days 30°C: 4 Days	60–70 days, 6–9 generations/year pupal hibernation
Musca autumnalis, face fly	5–7	600–900 on feces	Temperature dependent	4–7 Generations per year	4–7 Generations per year	♀ of last generations hibernates
Fannia canicularis, small sink fly	4–6	Feces and putrescent material	25°C: 20–48 h	6 Days	7–10 Days	6–7 Generations per year
Muscina stabulans, false stable fly	6–8	Eggs, larvae on chicken feces	Life cycle in summer about 2–3 weeks	Life cycle in summer about 2–3 weeks	Life cycle in summer about 2–3 weeks	4–5 Generations per year
Stomoxys calcitrans, biting stable fly	6–7	800 in groups of 25–50 in silage, in stables with urine and feces	1–2 Days, temperature dependent: 14 days up to months	6–8 Days, temperature dependent: 14 days up to months	6–8 Days, temperature dependent: 14 days up to months	♀ Live about 70–90 days
Haematobia irritans, small stable fly	4.5–4.5	In fresh cattle dung	Temperature dependent: 24 days up to months, optimum 27–30°C	Temperature dependent: 24 days up to months, optimum 27–30°C	Temperature dependent: 24 days up to months, optimum 27–30°C	3–4 Generations per year
Calliphora spp., blue blowfly	9–14	Eggs on feces with cadavers	Temperature dependent	Temperature dependent	10–40 Days	1–2 Months
Sarcophaga carnaria, gray flesh fly	10–19	Eggs on cadavers, agent of myiasis	After deposition	Temperature dependent	10–40 Days	1–2 Months
Lucilia sericata, green blowfly	5–11	1,000–2,000 eggs in batches of 250 on feces, wounds, meat	16–24 h	4–7 Days	1–3 Weeks on the ground	1–2 Months
Oestrus ovis , sheep nose bot fly	8–15	8–10 larvae are deposited at the nose or eyes	Immediately after laying	Larvae hibernate in the nose	2–4 Weeks on the ground	4 Weeks
Hypoderma bovis, warble fly	13–15	600–800 eggs on hair	4–7 days, then invading the skin	Inside the body until March	15–65 Days on the ground	3–5 Days
Hypoderma lineatum, cattle grub fly	11–13	5–20 eggs per hair	3–6 days, then invading the skin	Inside the body until March	23–28 Days on the ground	3–5 Days

Table 14.2 Transmission of agents of diseases and/or introduction of diseases by flies and other ectoparasitic insects (examples)

Ectoparasites	Symptoms of disease
House flies*: Musca domestica,* etc.	Mechanical vector for more than 100 animal and human pathogenic viruses, bacteria, and parasites. In case of ruminants, the following symptoms may occur besides restlessness, reduced food uptake, and even loss of weight: mastitis, diarrhea, and eye diseases due to bacteria and *Thelazia* worms
Cadaver flies*: Calliphora, Lucilia* species, *Sarcophaga*	Myiasis in wounds or in the hair and nostrils; transportation of viruses, bacteria, or parasitic eggs/larvae to mouth, eye, udder, or wounds
Stable flies*: Stomoxys calcitrans*	Inflammation of biting sites, restlessness, hypersensitivity, anemia, loss of weight, potential mechanical transmission of viruses or bacteria (e.g., paratyphus)
Bot flies*: Hypoderma* species*, Oestrus ovis*	Restlessness, hypodermosis, inner edema, paralysis during larval wandering, possible death, loss of the leather due to skin bots and nose bots, nose and eye problems, wrong turning syndrome in sheep, general loss of weight
Simuliids*: Simulium, Odagmia, Boophthora* species	Painful, burning bite sites with considerable subcutaneous hemorrhages, shock reaction in case of numerous bites, heart and blood circulation problems, paralysis of breathing activities, eventual death in case of mass infestation, transmission of filarial worms
Tabanids*: Tabanus, Haematopota* species	Painful bites introduce restlessness and severe itching and therefore loss of weight follows. Mechanical transmission of *Anaplasma* stages, bacteria, filarial worms, and probably also viruses
Blood-sucking lice*: Haematopinus, Linognathus* species	Restlessness, itching, loss of hair and weight, anemia, reduced activity
Mallophaga*: Bovicola, Lepikentron* species	Restlessness, itching, loss of hair, skin infections, loss of weight
Louse flies*: Lipoptena, Melophagus* species	Restlessness, itching, loss of hair and weight, dermal myiasis
Mosquitoes*: Aedes, Cules, Anopheles* species etc.	Itching, potential transmission of viruses, bacteria, protozoans and worms, restlessness and skin edema
Midges*: Culicoides* species	Vectors of Bluetongue and other viruses, possible death, painful bites, skin edema, especially at the udder, loss of weight, restlessness

14.2.2 The Secondary Healing Wound

This process occurs in superinfected, larger wounds and includes also all "nonhealing wounds" such as decubitus, diabetic foot, ulcus cruris etc.

In many cases the *primary* and *secondary wound-healing* process covers three, sometimes overlapping phases.

Table 14.3 List of bacteria and fungi found on the mouthparts and other regions of the body of different flies caught close to farms (according to Förster et al. 2007)

Microorganisms isolated	Test flies (No)	Percent ($n = 56$)
Bacteria		
Acinetobacter wolfii	1	1.8
Aerobic spore formers	49	87.5
Corynebacteria	7	12.5
E. coli	15	26.8
EAEC	1	1.8
EPEC	1	1.8
ETEC	2	3.6
Enterobacter aerogenes	3	5.4
Enterobacter group	9	16.1
Enterococci	3	5.4
Enterococcus faecium	1	1.8
Klebsiella spp.	3	5.4
Morganella morganii	7	12.5
Meisseria sp.	4	7.1
Nonfermenter group	1	1.8
Pantoea agglomerans	1	1.8
Proteus sp.	20	35.7
Providencia rettgeri	4	7.1
Pseudomonas sp.	1	1.8
Sphingomonas paucimobilis	1	1.8
Staphylococci, coagulase-negative	20	35.7
Staphylococcus aureus	5	8.9
Streptococcus sp.	5	8.9
Streptococcus viridans group	10	17.9
Fungi		
Aspergillus fumigatus	1	1.8
Mucor sp.	1	1.8

No. Number of test flies carrying microorganism; *Percent* percentages of total test flies transferring microorganisms ($N = 56$)

14.2.2.1 Phase of Cleaning, Exudation, and Inflammatory Reactions

This phase starts seconds after the injury by bleeding which removes remnants of cells and/or bacteria etc. The body contracts blood vessels in order to decrease the loss of blood. Contact with oxygen and release of tissue factors activating factor VII introduces the chain of the blood coagulation cascade, which is accompanied by the production of growth factors, cytokines, and installation of the arachidonic acid pathway. The visible symptoms of these processes are occurrence of heat, redness, and swelling along the wound region and feeling of pain (classically described as *rubor*, *calor*, *tumor*, and *dolor*). These phenomena and the final formation of a fibrin clot closing the wound support the *hemostasis*. Besides their main function during hemostasis the thrombocytes (= platelets) release many proinflammatory peptides (e.g., TGF-β, PDGF), which act as chemoattractants for monocytes, neutrophilic cells, and fibroblasts. At the same time the damaged tissue releases arachidonic acids (= fatty acids from membranes) and eicosanoids. Both stimulate the immune reaction. The release of Interleukin-2-cytokine by T-cells thus promotes the mitosis

and formation of monocytes, which have their maximum between 48 and 60 h after the wound appeared and which are finally transformed into macrophages. During the phagocytosis of bacteria and cell remnants the macrophages release a factor (bFGF), which acts as a chemoattractant for fibroblasts and endothelial cells, in addition to IL-1, which stimulates the division of many cells that are needed during angiogenesis. Finally, the macrophages excrete MDGF (Macrophage-Derived Growth Factor), which increases the migration of keratinocytes, fibroblasts, and endothelial cells into the wound. This phase is mostly finished within 3 days after the injury.

14.2.2.2 Phase of Granulation and Proliferation

This phase starts with the migration of the fibroblasts from the wound's border in the direction of the center of the wound, being attracted by PDGF, TGF-β, and bFGF factors in the wound's fluid. These fibroblasts release proteoglycans and glucosaminoglycans as a basis for a new extracellular matrix formed by collagen and granulation tissue. The number of fibroblasts reaches its maximum between the 7th and 14th day having started as early as on the 2nd day after injury. The newly biosynthesized collagen is then gathered together extracellularly by fibroblasts forming cross-linked fibers. This formation of a new connective tissue goes on continuously for 6 weeks, while endothelial cells and keratinocytes are also growing continuously. In parallel to these processes angiogenesis occurs developing new blood vessels from the edges of the wound into its center. The new vessels produce collagenase and plasminogen activators, which support the slow digestion of fibrin clots and of the temporary matrix, while further new granulation tissue (collagen, capillaries, and extracellular matrix) is formed to cover the whole wounded area. The degrading provisional matrix sets free hyaluronic acid, while the chondroin sulfate level decreases, which slows down fibroblast migration and proliferation. Since the tissues are well supplied with blood, the wound appears reddish and granulated. ´This phase therefore is also called the "granulation phase." The surface of this wound appears smooth, but it must be protected from mechanical injury and kept humid at any time.

14.2.2.3 Phase of Maturation, Regeneration, and Epithelization

This phase may start in wounds as early as on the 4th day and may need 21–25 days. In chronic wounds, however, it may take weeks, months or even years. The main process is the maturation of collagen. The primarily formed collagen type I is gradually replaced by collagen type III, which forms the scar. The granulation tissue loses water and epithelial cells protrude into the center of the wound and this layer becomes thickened. Then fibroblasts differentiate into myofibroblasts including α-smooth muscle actin fibrils, which bind cells together causing a buckling of the wound's edges which move closer together. These

myofibrils are responsible for wound contraction and apparently play a role as precursors of apoptosis, since finally the wound tissue is replaced by healthy tissue.

14.3 The "Nonhealing Wounds": The Problem

Skin is the most important barrier in protecting humans and animals from external environmental influences, for example it offers shelter against loss of temperature and toxic solutions as well as hindering or even blocking penetration of agents of diseases including ectoparasites. Openings in the body surface are entrance doors called wounds, which may lead to outflow of blood and lymph fluid. Thus, all living beings have developed sophisticated methods to close such wounds as soon as possible. In general – under normal conditions even without medical support – a wound heals at the latest within 13–30 days during which a cascade of events occurs including the healing phases of blood clotting, inflammation, tissue formation (= cell proliferation), and final remodeling. These different stages involve processes such as coagulation, prevention of infection, exudation, angiogenesis, collagen biosynthesis, and finally epithelization. This leads at the beginning to the formation of rather weak scar tissue without hair, follicles and glands, which later, however, will be re-formed.

According to the standards of the so-called "school medicine" there are several types or origins of wounds (Protz 2009; Knapp and Hansis 1999):

1. Mechanical wounds
2. Thermic wounds
3. Chemical wounds
4. Ulcus wounds

If these wounds do not heal within 4–12 weeks – even under strict clinical treatment – they are considered *chronic wounds*. Especially in group 4 of the wounds cited above three basic types can be diagnosed.

14.3.1 Decubitus Wounds

These wounds have their origin in pressure afflicting certain regions of the body due to immobility and due to several diseases such as diabetes, disturbances in metabolism, damage to blood vessels, drug abuse, dementia etc. According to the European Pressure Ulcer Advisory Panel (EPUAP) there are four grades with the following main characteristics:

1. Phase one: Skin reddening
2. Phase two: Partial loss of the skin

3. Phase three: Deep hollows in tissues
4. Phase four: Involvement of destruction of bones and muscles

14.3.2 Ulcus Cruris (venosum) Wounds

This disease is due to a chronic deficiency of the walls of the veins (very often the veins along the legs are afflicted). Such an ulcus is described as therapy resistant, if there is no healing within 1 year under use of approved clinical methods. Dermatologists differentiate three different grades:

1. Occurrence of edema on the legs (in the region of the knuckles)
2. Edema along the tibia, pigmentation of the skin, atrophies along the skin of the legs
3. Ulcus formation

14.3.3 Diabetic Foot Syndrome

About 20% of all patients suffering from the disease *diabetes mellitus* are finally hit by *diabetic foot syndrome*. This syndrome has as its origin in more than 50% of cases a so-called polyneuropathy, which is described as a deficiency of the sensoric, motoric, and autonomous nerves leading to the following symptoms:

1. *Sensoric neuropathy*:
   Appears as loss of sensitivity and/or occurrence of sudden itching of the sole of the foot.
2. *Motoric neuropathy*:
   The deformation of the muscles of the feet leads to a changing of the movement of the whole body thus introducing the increase of the stratum corneum along the sole of the feet (= appearance of a thick horny skin).
3. *Autonomous neuropathy*:
   This stage is introduced by a dilation of peripheral blood vessels. This leads to reddening and heating up of the skin. About 15% of cases of diabetic foot syndrome are due to an occlusion of the peripheral arterial blood vessels (the risk for diabetes patients is about six times higher than in normal persons). In general the diabetic foot syndrome occurs in more than 50% of cases due to the coexistence of all three forms of neuropathy.

The classical differentiations of the diabetic foot syndrome of *Armstrong* respectively *Wagner* (see Protz 2009) consider groups *A–D* (Armstrong) respectively 0–5 (Wagner). The most severe stage in both grading lists are characterized by a complete necrosis of the foot combined with local loss of blood supply and additional bacterial superinfection (Table 14.4).

Table 14.4 Factors that may make wounds "nonhealing wounds"

Type of factor	Effects
Necrotic tissue	Formation of a growth medium for bacteria increasing their number to more than 10^5/g tissue
Bacterial infection	Slow down of restoration of the wound
Degradation of cells	Slow down of cell migration
Increase of proteases	Toxic effects on healthy cells, which may die or reduce migration into wounds, granulation is retarded
Destruction of blood vessels	Macrophages and own proteolytic enzymes do not arrive in wounds, oxygen cannot arrive and decreases in wounds thus slowing down formation of collagen, lack of oxygen (less than 40 mmHg), blocks linking of fibrils due to blocking of hydralization of lysine and proline, which are the main amino acids of collagen
Lack of nutrients due to insufficient transport	Lack of fatty acids leads to reduced membrane formations, lack of amino acids reduce enzyme and protein formation, lack of vitamin K causes coagulopathy lack of metals (calcium, copper, iron, zinc etc.), reduce protein synthesis

14.4 Available Approaches to Heal "Nonhealing Wounds"

14.4.1 Mechanical Remedies

In traditional wound care the following remedies are used (Table 14.5):

(a) Compressions
   1. Mull covers
   2. Fleece covers
   3. Plaster, pavements
   4. Covers containing elements of polyurethane, silver, alginates, hydrocolloids, active charcoal, collagen, betaisadonna etc. (Hoffman 2002)

(b) Medicinal honey covering the wound

14.4.2 Chemotherapeutics

The use of wound-rinsing baths (hydrotherapy) is common, but depends on the stage of the wound. Sterile fluids containing chemotherapeutically active substances are used (e.g. polyhexanid, antibiotics, jodids, or even H_2O_2). Furthermore, enzymes are used to obtain a debridement of the borders of the wound – often in combination with systemically acting antibiotics. Recently also wound dressing containing growth factors have been in use.

The *enzyme therapy* is rather old, since in former times enzymes used were often obtained from fruit juices (e.g. lemon). However, although the efficacy of such juices had been approved in tests since 1960, for the past 10 years proteolytic enzymes have been preferred and were adapted as standard methods for necrotic

Table 14.5 Examples of wound dressings that claim to influence wound healing

Type of dressing	Mechanism of action	Authors
Addition of honey (Manuka honey)	Changing of pH on the wound acts via lowering activity of wound proteases	Gethin et al. (2008)
Alginate containing dressings with uron acids and polysaccharides	Stimulated granulation processes of the wound	Lee et al. (2009), Murakami et al. (2010)
Alginate containing wound dressing	Binding of proinflammatory factors	Wiegand et al. (2009a, b)
Natural extracts and Medihoney	Claim influences of antibacterial compounds	Jull et al. (2008), Robson et al. (2009), Blair et al. (2009), George and Cutting (2007)
Polymer and gel dressings	Managing of wound exudate levels by protecting against wound dehydration and external bacterial contamination, producing cushioning and absorption	Many products; e.g. Principelle Matrix® Fa. Principelle BV, The Netherlands

tissue removal. Furthermore, streptokinase (originating from nonpathogenic *Streptococcus* group C) is used to cut proteins and peptides. This enzyme also catalyzes the production of plasmine from plasminogen. The plasmine degrades blood clots by fibrin digestion and prevents the new formation of fibrin by decomposing fibrinogen, factor V and VII into peptides and amino acids. Another enzyme used is streptodornase which is produced by fermentation of *Streptococcus haemolyticus* products. Other enzymes in use are desoxyribonuclease, fibronilysine, krillase, collagenases of human type (elastase, hydroxylase, myeloperoxidase), and bacterial collagenases [e.g., from *Clostridium histolyticum*] belong also to the spectrum of remedies (Huberman et al. 2007; König et al. 2005; Lappin-Scott 1998).

14.4.3 Debridement by Surgery

Surgical debridement is carried out with the help of the good old scalpel and leads to the removal of necrotic layers. This result can also be obtained by an ultrasound therapy in a waterbath or via a hydrogel dressing. Furthermore, a laser and beam may be used, too.

14.4.4 Maggot Biotherapy

Maggot therapy (see Chap. 13 of this book) is very old and was already used by the early high cultures of the Maya up to the Australian aborigines of today. In the

Medieval wars of the knights and in the European wars (around 1800) with and against the French usurper Napoleon Bonaparte, medical doctors observed that the wounds of soldiers had different consequences depending on the situation, whether they were infested with fly larvae or not. Unexpectedly those soldiers survived whose wounds contained fly larvae, while those without larvae often died. This knowledge was rediscovered in the First World War (1914–1918) and used until the penicillin antibiotics were discovered (Baer 1931).

In the early 1980s, this form of biosurgery was again rediscovered and is used today in Germany as a so-called *"individually prescribed medicament*." About 5–8 larvae per centimeter are placed within a gauze-bag onto the wound. After 2–4 days the bag is taken away and the wound is rinsed to remove potential bacterial super-infections. It is claimed that the excreted proteinases of the salivary glands lead to disinfection of the wound and to its debridement by dissolving dead cells and thus allowing healthy intact tissues to grow again and to cover the wound surface (e.g., comments of the producers – Fa. Biomonde and Fa. Agiltera, Germany). However, many of the different efficacies of the maggots are not very clearly documented, but in any case wound healing works in many cases of so-called "nonhealing wounds." If this interpretation is true, the effects are due to pharmacological reactions and thus the flies are *"living medicaments"* [Figs. 14.1–14.9 (for figures 14.2, 14.3, 14.7 and 14.8 see end of this chapter)].

14.4.5 Osmotic Debridement

This method is one of the oldest ones, which applies hyperosmolar sugar derivates such as diextranomer (e.g., as honey) twice a day. This procedure binds fluids and acts antiseptically. However, there may be allergic reactions and gluing of the wound surface (Table 14.5, Manuka- and Medo-honey).

14.5 The "Other" Approaches: Extracts to the Fore

14.5.1 The Idea – Why Use Extracts of Fly Larvae

When looking at the present situation of increasing numbers of diseased elderly people suffering from "nonhealing wounds" due to different supporting factors and the fact that severe resistances against antibiotics have been developed by many bacteria, the method involving the use of fly maggots for biosurgery represents important progress. However, this method has also some weak points:

1. Many people are disgusted with the idea that living fly larvae are feeding on their body.
2. The larvae move constantly on the wounds. Thus, there is a permanent irritation due to their movements and attachment of their mouth hooks [Figs. 14.3–14.5

(for figure 14.3 see end of this chapter)], although they are embedded in bags of gauze. These movements remind patients all day and night of the presence of the fly larvae.
3. Living fly larvae digest and thus excrete feces, which in combination with the fluid on the surface of the wounds produces a "somewhat muddy smell." Of course these feces lead also to a certain nonsterility.
4. The pads containing the fly larvae cannot be stored but must be constantly reproduced, since larvae two of *Lucilia sericata* are used in the bags, which will pupate after another molt at the latest within 3–5 days. This inevitable process may lead to logistic problems, since the pads have to arrive always just in time in order to be put to use for a period of about 3–5 days at the maximum.

Therefore, the idea to examine extracts of fly larvae was born in several groups of researchers (Bowles et al. 1988, 1992, 1996; Casu et al. 2000; Cerovský et al. 2010; Chambers et al. 2003; Chapman 1997; Elkington et al. 2009; Foti et al. 2007; Greener et al. 2005; Harris et al. 2009; Kerlin and East 1991; Light et al. 2000; Nemoto et al. 2003; Prete 1997; Tellam et al. 2001; Wicke et al. 2000; Wollina et al. 2002; Ziffren et al. 1953). This idea was primarily based on the morphology of these fly larvae. Fly larvae have neither chewing mouthparts, that would allow them to bite off pieces of dead tissues (as was formerly erroneously believed) nor do they possess any other true mouthparts. The dense structure seen at the anterior end of the larvae (Fig. 14.4) represent two hooks (Fig. 14.5), which are used as holdfast systems while the larva migrates forward by body stretching movements on the surface of wounds or meat (in cultures). The feeding process of fly larvae occurs by repeated sucking of portions of the fluid from the surface of wounds after they had secreted large amounts of saliva and intestinal fluid onto the wound. The larval excretions become mixed with fluid from the surface containing apparently lysed tissue materials.

This behavior and the effects seen along the wounds nourished the suspicion that the salivary excretions contain defined active compounds that might be extractable by various standard laboratory methods in order to become stored, for example after lyophilization. Therefore, it was not surprising that several groups started research in this direction. However, this turned out to become a promising path, since there are no animal models for testing the wound-healing process that should go on in the "nonhealing wounds" of humans.

14.5.2 The Life Cycle of the Fly Lucilia sericata

The so-called gold-fly or green-bottle *L.* (syn. *Phaenicia*) *sericata* [Fig. 14.8 (for figure see end of this chapter)] belongs to the family of Calliphoridae (blow-flies). *L. sericata* occurs in many temperate climates including Australia, New Zealand, Northern Europe, and North America. The body of the adults appears robust and has a length of about 6–9 mm. Thorax and abdomen appear bright metallic green.

The palps are yellowish. The dorsal thorax has no stripes and the bristles are stout. The dorsal side of the abdomen is characterized by a faint longitudinal line along the middle [Fig. 14.8 (for figure see end of this chapter)]. After copulation the females deposit about 10–15 times batches of 100–200 whitish eggs on substrates [Fig. 14.2 (for figure see end of this chapter)]. Apparently this species prefers sticky wounds for its egg deposition, since Martini (1946) reported that in 43 cases of *Lucilia* myiasis larvae were found on the following wounds:

17 cases in tuberculosis open bones
5 cases of infected ulcera
6 cases of syphilitic wounds
5 cases of lupus
5 cases of infected skin lesions
5 cases of chancroids
2 cases of oriental sore (due to *Leishmania* spp.)
1 case of skin cancer
1 case of lichen infection
1 case of mastitis
1 case of hemorrhoidal sore
1 case of blastomycosis
2 cases of framboesian ulcer
But only 1 case of a normal wound due to a knife-cut

In the laboratory (we use horse-meat in our laboratory) the development from egg laying until pupation takes about 6–7 days under standardized temperatures of 27°C. The larva one (maggot) has a length of about 1–2 mm when hatching within 24 h from the egg. After feeding sufficiently larva three gains 100 times more weight and reaches a length of 8–12 mm [Fig. 14.3 (for figure see end of this chapter)]. Then it leaves the feeding substrate and creeps for pupation to dry and hidden places, where the formation of the adult female or male takes place inside the pupal cocoon [Fig. 14.7 (for figure see end of this chapter)]. Depending on the temperature this process needs in general about 2 weeks. The adults have a life span of about 30–45 days.

14.5.3 Types of Extracts of Fly Maggots

14.5.3.1 Extracts of Salivary and Intestinal Excretions of *L. sericata* Maggots

As listed in Table 14.6 there have been many attempts to use different extracts from fly maggots for products to heal "nonhealing-wounds." Since wound healing is a very complex process of different steps in a broad cascade of events, the idea is that the addition of some blocked factors or the "deblocking" of blocked factors as well as the stimulation of particular activities may be successful with respect to causing

a return to the events and steps of normal wound healing. Because of the complexity of the wound-healing system, of course several approaches will lead to more or less significant healing effects. The effects described when using the different extracts listed in Table 14.6 vary often considerably. This fact is very probably based on the great variation of "nonhealing-wounds": not one is identical to the other (Table 14.4).

Table 14.6 Compounds that are or might be used in would healing

Type of extract	Mechanism of activity	Authors
Excretory/secretory extracts in PBS	Motogenic activity on fibroblasts, determined in cell culture	Smith et al. (2006)
Extract contains essential amino acids	Change of microenvironment	Cassino and Ricci (2010)
Extract contains collagen, elastin, glycosaminglycans, glycoproteins, proteoglycans (OASIS product)	Formation of a cover, change of microenvironment	Romanelli et al. (2010)
Extract contains collagen type 1	Binds and inactivates proinflammatory cytokines and proteases	Wiegand et al. (2009a, b)
Extract contains collagen type 1	Deactivation of proteases in the fluid of the wound	Smeets et al. (2008), Lobmann et al. (2006)
Extract contains collagen	Change of microenvironment, better microcirculation due to covering the wound	Andriessen et al. (2009)
Extract interacts with angiostatin and other proteases	Binding and inactivation of angiostatin (= Kringle 1–3 from plasminogen), that acts negatively on formation of blood vessels and growth factors	Takahashi et al. (2010), Patthy et al. (1984), Aisina et al. (2009)
Extract of salivary glands	Prohibition of the formation of proinflammatory factors by neutrophilic granulocytes	Pecivova et al. (2008)
Secretions of fly maggots	Support of the development of monocytes into macrophages, change of microenvironment from proinflammatory to proangionenic	van der Plas et al. (2008, 2009, 2010)
Full extract of squeezed pupae (heated on 65°C)	Forms together with the fluid on the wounds a fine film which becomes attached to the wound dressing and thus removes inflammatory factors from the wound	Patents of the researcher group of Fa. Alpha-Biocare, Germany
Full extracts of squeezed larvae (heated on 65°C)	Forms together with the fluid on the wounds a fine film which becomes attached to the wound dressing and thus removes inflammatory factors and potential bacteria from the wound's surface	Patents of the researcher group of Fa. Alpha-Biocare, Germany

14.5.3.2 Extracts of Whole Pupae and Larvae of *L. sericata*

In our laboratory aqueous extracts were made from larvae respectively pupae after intense cleaning of the surfaces and after homogenization of the whole individuals.

After filtration this homogenate was heated for *x* min at 65°C leading to a fall-out of rough material. The remnant fine homogenate was stepwise ultrafiltrated, sterile filtered, and subsequently transferred into vials that were subjected to lyophilization and firmly closed. Prior to use this ultrafiltrate was diluted in a physiological fluid and then used for different trials. For exclusion of any secondary infection the vials had been radiated previously with γ-rays. The whole system was submitted to the process of obtaining patents. Some of which had been granted in the mean time. While testing the extract – besides many other trials and tests – it was seen that the extract upon contact with the fluids of the surface of "nonhealing-wounds" formed a fine film [Figs. 14.10–14.15 (for figures see end of this chapter)], which will be surely attached to the wound dressing and thus will be removed, as the normal wound dressing is regularly changed. This effect was apparently the basis of the debridement seen in some cases of so-called "healing trials" in persons, which had not reacted to any other medication. After repeated use of this procedure the debridement was successful in that the wounds were nearly closed 6–8 weeks after the first application of the extract [Figs. 14.16 and 14.17 (for figures see end of this chapter)]. This lyophilized extract is now registered under the name "Larveel®." The product removes after application apparently mechanically the proinflammatory substances from the wound's surface thus changing the microenvironment of the wound, so that normal autolysis of necrotic tissues by the wound itself may start again. Of course also the number of bacteria, which may have developed on the wound, is constantly reduced during each changing of the wound dressing, since these bacteria are apparently included in the fine films formed on the wound.

14.5.4 Characteristics of Larveel®

The extract has the following properties:

1. It is an aqueous, ultrafiltrated extract of whole maggots of larvae and/or fresh pupae of *L. sericata*.
2. It is heated at 65°C leading to a fall out of dense material.
3. It is absolute sterile.
4. It does not contain compounds for conservation.
5. It does not contain chemical ingredients other than those coming from the fly stage.
6. Thus it is a completely natural product.
7. In skin tests with daily doubling of doses no irritation was noted on the skin of test persons.

8. It produces a fine film on the wound exudates, which becomes attached to the normal wound dressing and thus apparently removes substances that block the normal debridement of the wound.
9. In healing trials very promising results were seen in finally healing "nonhealing wounds."

14.5.5 Advantages of Larveel® Versus Living Maggot Therapy

1. Patients treated with living maggots feel psychological stress when thinking that "insects are feeding on their wounds."
   Larveel® is used like a normal wound dressing.
2. Living larvae move and this is noted by the patient leading to restlessness.
   In Larveel® treatment no movements occur on the wound.
3. Living larvae have intestinal excretions (since they increase their body weight up to 100 times). These excretions introduce a somewhat "muddy" smell to the wound.
   Larveel® has no smell.
4. Living larvae grow every day. Therefore, they can only be used at a certain stage and thus they cannot be stored in pharmacies.
   Larveel® can easily be stored.
5. Because of the need that living larvae must be used at a certain stage, logistic problems during transportation may occur.
   Larveel® can be stored for long periods at adequate temperatures, it is available on request.
6. Living larvae – although widely produced sterile – will become infected with bacteria/viruses from the wound and perhaps support an increase in their numbers.
   Larveel® diminishes the amount of bacteria, which are normally on a wound, by including them into the fine film formed on the wound, which is removed during the changing of the wound dressing.
7. The costs of the use of living larvae must be rather high due to the sterile production and fresh transportation.
   Larveel® is more economical to its rather easy production in larger lots.

14.6 Conclusions

The present and the preceding chapters show that nature has developed many methods to protect individuals from death. All these methods were developed for survival in the struggle for life during evolution. Today we only see those organisms that were successful in this fight for survival. Therefore, it is the task of scientists to determine what can be used from these successful "experiments of

nature." Some of these "natural inventions" had been noted by mankind even a thousand years ago by empirical observation. Such experiments as the obvious benefits of otherwise mostly nasty flies with respect to wound healing led to their use already in the early history of mankind. However, this knowledge was often forgotten, but also often rediscovered, so that today the use of larvae and/or the use of extracts of larvae and pupae really offer progress in the treating of "nonhealing wounds" in times of society-based diseases.

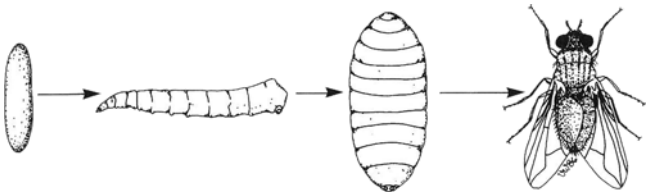

Fig. 14.1 Diagrammatic representation of the life cycle stages of flies (there are three larval stages, which grow via two molts)

Fig. 14.2 Light micrograph of a batch of *Lucilia sericata* eggs

Fig. 14.3 Light micrograph of a third stage larva of *L. sericata*. Note the dense appearing mouth hooks

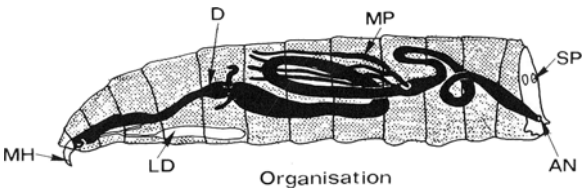

Fig. 14.4 Diagrammatic representation of a fly larva showing intestine and labial = salivary glands. AN = anus, D = gut, LD = labial glands, MH = mouth hooks, MP = malpighi ducts, SP = spiracles

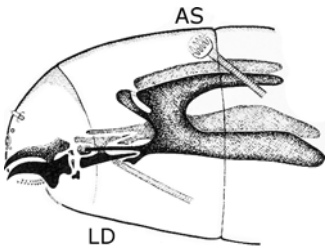

Fig. 14.5 Diagrammatic representations of the mouth hooks. AS = anterior spiracle, LD = labial duct starting from mouth

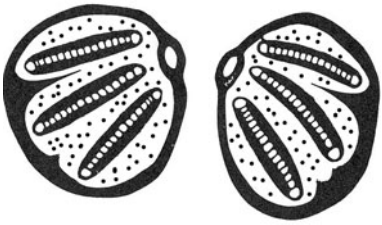

Fig. 14.6 Diagrammatic representation of the appearance of the two abdominal spiracles of larva three

Fig. 14.7 Light micrograph of a pupae of *L. sericata*

Fig. 14.8 Adult *Lucilia sericata*

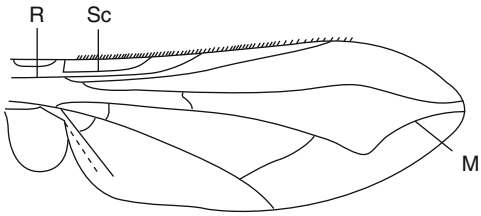

Fig. 14.9 Diagrammatic representation of a wing of an adult *Lucilia sericata* (letters M, R, Sc mark important “veins” of the wing)

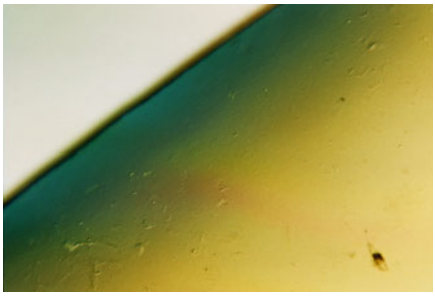

Fig. 14.10 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 1)

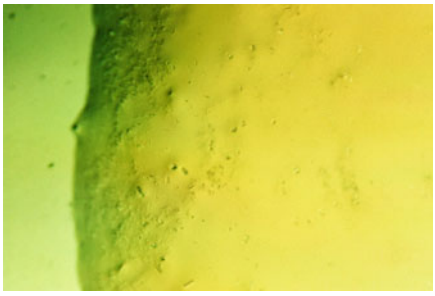

Fig. 14.11 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 2)

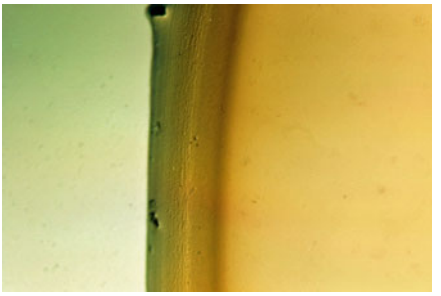

Fig. 14.12 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 3)

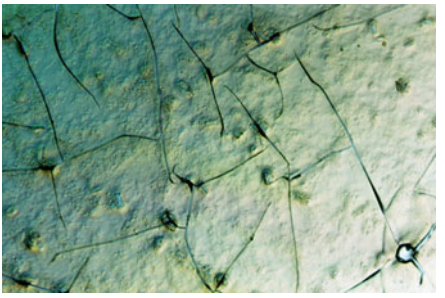

Fig. 14.13 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 4)

Fig. 14.14 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 5)

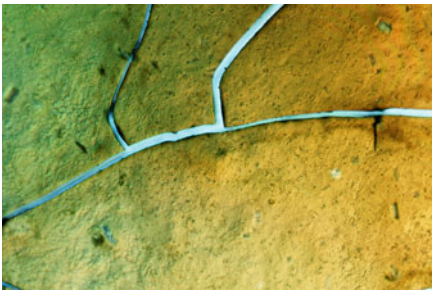

Fig. 14.15 Formation of a fine film on a wound exudate after application of the larval extract in six steps (step 6)

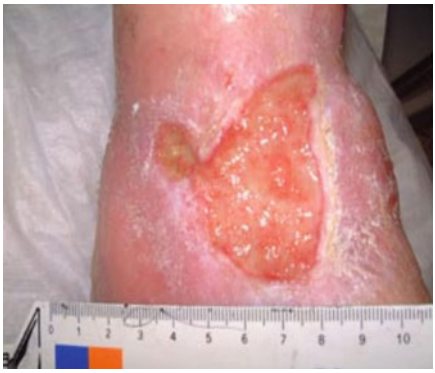

Fig. 14.16 Nonhealing wound of a patient before the application of the extract (photo by Prof. Dr. Stege from a patient application, given to Fa. Alpha-Biocare)

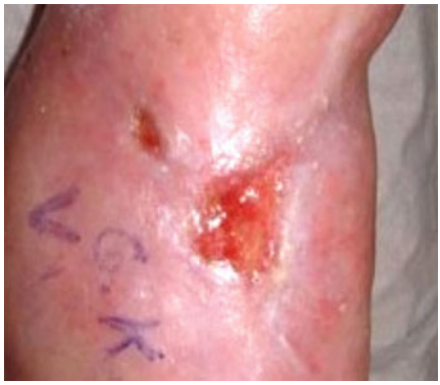

Fig. 14.17 Wound 6 weeks after application of the extract. Note the tendency to close the surface (photo by Prof. Dr. Stege given to Fa. Alpha-Biocare for a common patent)

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